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Undifferentiated Rhabdomyosarcoma With Lymphoid Phenotype Expression

Alfredo Pinto, MD,[1]* Giovanni Tallini, MD,[2] Robert W. Novak, MD,[3] Tom Bowen, MD,[4] and David M. Parham, MD[5]

Poorly differentiated rhabdomyosarcomas are traditionally distinguished from lymphomas by their absence of lymphoid markers such as immunoglobulin or CD20 expression. We have encountered three alveolar rhabdomyosarcomas that were initially diagnosed as lymphoid neoplasms because of the expression of a lymphocytic phenotype in morphologically undifferentiated tumor cells. Subsequent cytogenetic analysis revealed a t(2;13) in two cases. All cases recurred in the chest wall and showed positivity for muscle markers, such as muscle-specific actin, myoglobin, MyoD1, and/or desmin on subsequent immunohistochemistry. The findings in these three cases lead us to conclude that the presence of a lymphoid phenotype does not absolutely exclude the diagnosis of rhabdomyosarcoma. **Med. Pediatr. Oncol. 28:165–170**

Key words: rhabdomyosarcoma; lymphoid phenotype; CD20; CD19; immunoglobulin

INTRODUCTION

Small round cell tumors in children (SRCTC) pose challenging diagnostic problems to the surgical pathologist, particularly if they are very undifferentiated. In view of the profound therapeutic implications, it is imperative that every effort be made to arrive at a specific diagnosis. Despite the use of electron microscopy and the development of an increasing number of antibodies that detect muscle-specific markers, the diagnosis of poorly differentiated rhabdomyosarcoma (RMS) is often difficult.

In particular, one SRCTC that must be distinguished from RMS is lymphoma. This distinction is made more difficult by the occurrence of RMS in a wide variety of sites, its common metastasis to regional lymph nodes, and its occasional presentation as widely disseminated disease with no apparent primary site [1]. Conversely, lymphomas may present in a soft tissue location [2]. Poorly differentiated RMS are traditionally distinguished from lymphomas by their absence of lymphoid markers. We describe three alveolar RMS that were initially diagnosed as lymphoid neoplasms because of the expression of a lymphoid phenotype in morphologically undifferentiated tumor cells.

CASE REPORTS

Patient 1

This 4-month-old boy was brought to the hospital because the parents noted a distended abdomen. Computed tomography of the abdomen revealed a large ($11 \times 11 \times 9$ cm) peritoneal mass. The patient underwent a debulking procedure. The tumor was diagnosed as malignant lymphoma, large cell type, on the basis of positivity for immunoglobulin (Ig)M, Kappa light chain, CD10, and CD24. All other markers including desmin, actin-HHF35, alpha smooth muscle actin, and myoglobin were negative. The patient was treated with combined chemotherapy with vincristine, doxorubicin, and prednisone. Four months after the diagnosis, the tumor relapsed at the primary site and was debulked again. It was histologically identical to the previous material, so immunostains were not repeated. The patient was treated with ifosfamide, carboplatin, and etoposide. After completion of this course of chemotherapy, a persistent $3 \times 3 \times 2$ cm mass was resected, which revealed a rhabdomyosarcoma with well-developed rhabdomyoblasts. A second relapse occurred 9 months after diagnosis and was treated with chemotherapy including vincristine, dactinomycin, cisplatin, and cyclophosphamide. There was an initial response followed by progressive tumor growth, leading to the death of the patient approximately 15 months after the original diagnosis.

Patient 2

This 14-year-old girl presented for evaluation of persistent cough, fatigue, weight loss, anorexia, and headache

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[1]Alberta Children's Hospital and University of Calgary, Calgary, Alberta; [2]Yale University, New Haven, Connecticut; [3]Children's Hospital Medical Centre of Akron, Akron, Ohio; [4]Victoria, British Columbia; [5]Arkansas Children's Hospital, Little Rock, Arkansas.

*Correspondence to: A. Pinto, MD, Clinical Laboratory, Alberta Children's Hospital, 1820 Richmand Road S.W., Calgary, Alberta, Canada, T2T 5C7.

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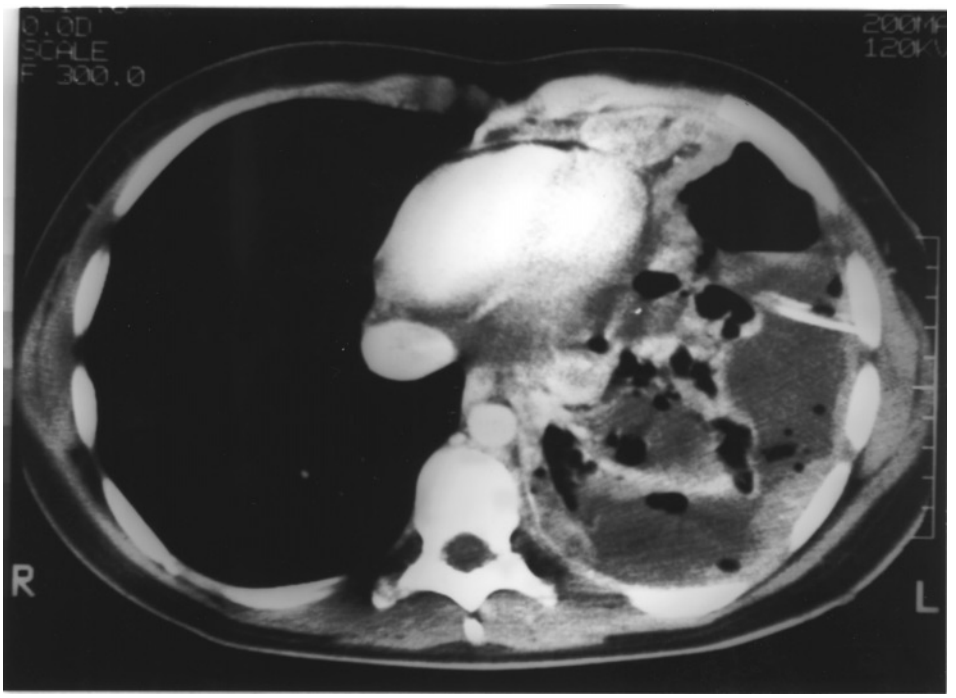

Fig. 1. Computed tomography scan in Patient 2 showing a left large pleural mass with compression of the lung. Used with permission (30).

for 2 months. Laboratory investigations revealed a leukocyte count of 8.8×10^9/l, hemoglobin of 106 g/l, platelet count of 335×10^9/l, serum lactate dehydrogenase of 7,975 u/l, and serum uric acid of 416 μmol/l. Computed tomography of the chest and abdomen revealed a large, left-sided, pleural-based soft tissue mass with massive pleural effusion, compression of the lungs, and displacement of the trachea and mediastinal structures to the right (Fig. 1). The diaphragm was difficult to visualize due to extension of the tumor. In the abdomen, retroperitoneal adenopathy surrounded the celiac axis and mesenteric vessels, interposed between the aorta and inferior vena cava, and was associated with massive ascites. Cytologic examination of the pleural fluid revealed malignant undifferentiated cells consistent with a B-cell lymphoma (Fig. 2A). Cytogenetic studies revealed no metaphases. Bone marrow examination was negative for tumor. The critical condition of the patient demanded rapid intervention and precluded further studies. The pleural cytologic preparation was reviewed by an expert consultant hematopathologist who agreed with the diagnosis of malignant lymphoma, small noncleaved cell type.

This patient was treated with a Pediatric Oncology Group protocol for B-cell lymphoma, Stage III. Six months after beginning therapy, she relapsed locally in the pleura and underwent an allogenic sibling bone marrow transplantation. At the time of relapse, a pleural biopsy revealed a primitive small round cell tumor (Fig. 2B). Tissue immunostains gave positive results with antibodies against Kappa light chain and CD20 and negative results with desmin antibody. Cytogenetic studies revealed 47XX, t(2;13) (q36;q14), +der (13), t(2;13) (q36; q14). Neither electron microscopy nor flow cytometry was performed due to the small size of the biopsy sample. Review of the second biopsy sample was performed by expert consultant hematopathologists, who again concurred with the diagnosis of malignant lymphoma, small noncleaved cell type.

Five months after the bone marrow transplantation, a relapse in the left pleural cavity was detected. A biopsy revealed an unequivocal rhabdomyosarcoma by light and electron microscopy (Fig. 3, A,B), and the karyotype revealed a t(2;13)(q36;q14) (Fig. 3C). Flow cytometric studies were limited because of the small biopsy samples but revealed tumor cells staining with both CD19 and Kappa.

Patient 3

This 14-year-old girl presented for evaluation of weakness and weight loss for 6 weeks. Laboratory investigations demonstrated a hemoglobin of 90 g/l, leukocyte count of 2.3×10^9/l, and platelet count of 89×10^9/l with scattered circulating atypical cells. Chest radiographs demonstrated modest mediastinal adenopathy. On bone marrow aspiration, more than 95% of the nucleated cells were found to be primitive, malignant-appearing cells with large irregular nuclei, prominent nucleoli, and abundant vacuolated basophilic cytoplasm. Occasional cells showed phagocytic activity. Periodic acid-shiff, methyl green-pyronin, and lymphocyte acid phosphatase stains showed block positivity; peroxidase, sudan black, and combined esterases were negative. Flow cytometric stud-

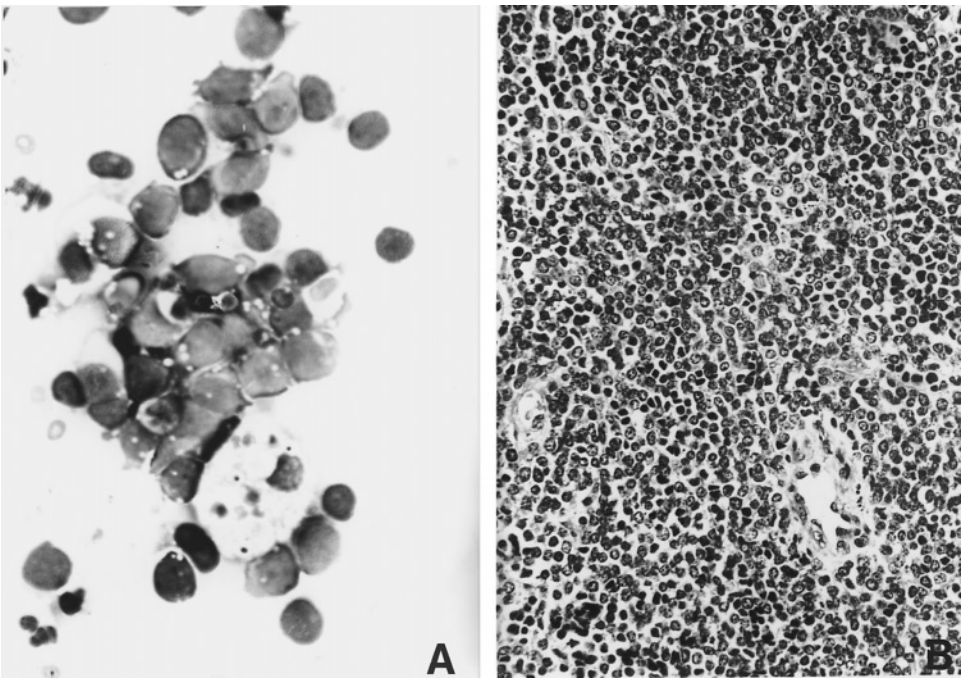

Fig. 2. Patient 2. **(A)** At diagnosis, cytology of the pleural fluid showed undifferentiated small round tumor cells with small cytoplasmic vacuoles. Wright-Giemsa, X1000. **(B)** At recurrence, pleural biopsy showed a patternless undifferentiated small round cell tumor. Hematoxylin-eosin X250.

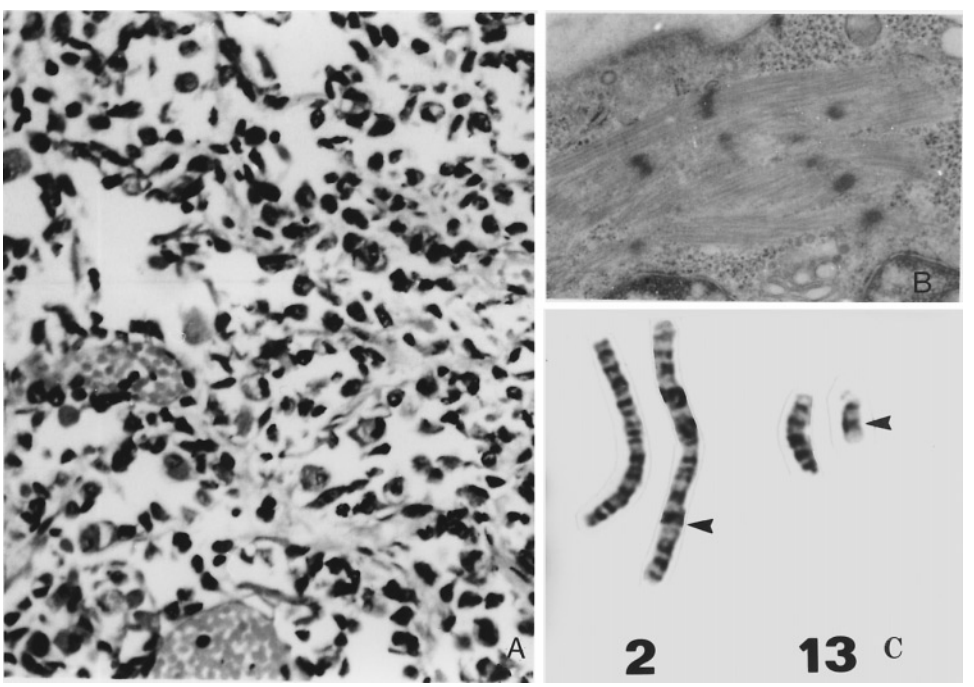

Fig. 3. Patient 2. After therapy, the tumor showed **(A)** cells with ample eosinophilic cytoplasm and pseudoalveolar pattern consistent with the diagnosis of alveolar rhabdomyosarcoma. Hematoxylin-eosin, X100. **(B)** Electronmicrograph showing z-band formation. Uranyl acetate-lead citrate X17000. **(C)** The t(2;13) reciprocal translocation with break points at 2q35 and 13q14. Used with permission (30).

ies demonstrated staining of primitive cells with CD20 only; CD2, CD3, CD10, CD19, and myeloid markers were negative. Electron microscopic examination revealed features compatible with primitive hematopoietic cells. Cerebrospinal fluid studies were normal. The patient began multidrug induction therapy for undifferentiated leukemia. Shortly after beginning therapy, a karyotype report showed cells with 78–101 chromosomes with a modal number of 85, a der(1), der(x), 9p+, t(2;13)(q37;q14), and four marker chromosomes. One week after beginning therapy, the bone marrow was extremely hypocellular with no tumor cells seen. At 30

days, the patient had a "remission" marrow. Central nervous system prophylaxis, intensification therapy, and maintenance therapy followed, and the patient remained free of clinically evident disease for 15 months. At 16 months, a chest wall tumor was detected. A biopsy was performed and demonstrated subepidermal infiltration of sheets of poorly differentiated tumor cells with large hyperchromatic nuclei and moderate amounts of cytoplasm. Electron microscopic findings were similar to the previous study; no basal lamina, junctional structures, or thick and thin filaments were found. Immunohistochemistry showed CD57 (Leu 7) positivity and focal muscle-specific actin (HHF-35) positivity. Repeat karyotyping showed hyperdiploidy with a t(2;13) (q37; q14).

DISCUSSION

RMS comprise a biologically heterogenous group of childhood malignancies that are histologically characterized by varying degrees of differentiation, ranging from undifferentiated primitive mesenchymal cells to fetal myotubes. Many cases of RMS can be diagnosed by their light microscopic appearance. Because of the use of immunohistochemistry, the presence of histologically defined rhabdomyoblasts and cytoplasmic cross-striations are not considered essential for the diagnosis of RMS, which may show no overt myogenic differentiation and lack a classical embryonal or alveolar pattern, as with the cases described in this report. The precise diagnosis of RMS may thus be exceedingly difficult, and on occasion none of the usual morphologic criteria may suffice in reliably arriving at a correct diagnosis.

All three of the cases presented here were initially diagnosed as lymphoid neoplasms because of the expression of a lymphoid phenotype in morphologically undifferentiated tumor cells (Table I). After combination chemotherapy and subsequent recurrence, these cases were obvious differentiated RMS as determined by standard light microscopy. Cytologic and phenotypic differentiation after polychemotherapy in RMS is well recognized [3], supporting the hypothesis that childhood RMS recapitulates the normal development of skeletal muscle and that RMS are arrested somewhere along their pathway to maturation [4].

Just as muscle markers are used to define RMS, a variety of antibodies against hematopoietic cell surface proteins are used for the diagnosis of hematopoietic malignancies. These antibodies are defined by antigens categorized by cluster designation (CD) [5]. As a consequence of their characterization as antibody-defined cell surface structures, CD molecules are very heterogeneous and comprise membrane-bound enzymes, signal transducers, activation antigens, and adhesion molecules [6,7]. Expression of CD molecules has been identified in various extrahematopoietic cell types and tumors [7–14]. There have been attempts to classify SRCTC by the expression of leukocyte differentiation antigens (CD molecules) [5,12,15].

Mechtersheimer et al. [11,12] have shown that RMS might stain variably with CD9, CD10, and CD56 antibodies. In our cases, Patient 1 was positive for CD10 by tissue immunostains. Pilkington and Pallesen [15] reported frequent staining of small round cell tumors of childhood with CD9 and CD10 antibodies. It has been postulated that expression of CD9 molecules among various types of SRCTC might reflect differences in the adhesive properties of the respective tumor cells [12]. CD36 staining in SRCTC was reported as restricted to scattered rhabdomyoblasts of two RMS [12]. McDonnell et al. [8] reported the staining of a primitive sarcoma, possibly RMS, with monoclonal antibodies for common leukocyte antigen (CD45). Expression of natural-killer–cell associated antigens CD56 and CD57, which are known to contain sequence homologues to the neural cell adhesion molecule N-CAM, was found in RMS [10]. In our cases, Patient 3 was positive for CD57 by tissue immunostaining. It is speculated that CD56 and CD57 might act as oncodevelopmental antigens [10].

Immunohistochemical stains in tissue sections revealed positivity with CD24 in Patient 1. CD24 is a single chain glycoprotein with phosphoinositol-linked functional structure [16] and at least three different epitopes. CD24 is expressed on B-lineage cells, granulocytes [17], other extrahematopoietic tissues [7,11], and in neuroblastoma [11,12], but no staining was detected in Ewing's sarcoma [12]. The expression of CD24 in RMS is considered a rare event.

CD19 was positive in Patient 2 by flow cytometry. CD19 is a lineage-specific, 95,000 Mr glycoprotein expressed by early pre–B-cells from about the time of immunoglobulin heavy-chain rearrangement until plasma cell differentiation [18]. CD19-mediated signal transduction initiates a series of biologic responses that are likely to be of central importance to B-cell signaling and/or growth regulation and the development of humoral immune response [19]. Several members of the CD19 cell surface complex are expressed by nonhematopoietic cells, suggesting that similar regulatory processes may occur in other tissues [19]. Although the precise in vivo function of CD19 has not been definitively elucidated, it is speculated that in conjunction with surface Ig, CD19 complex can serve an accessory role to enhance antigen-driven B-cell activation [19].

Immunostaining with CD20 was positive in tissue sections (Patient 2) and flow cytometry (Patient 3). The CD20 cluster contains three antibodies: B1(B5), 2H7 (B22), and 1F5 (B24). The prototype of this group is the B1 antigen that defines a 35-kd nonglycosylated phosphoprotein [20]. CD20 expression is nearly identical to CD19 expression. CD20 antigen is B-lineage–restricted and expressed throughout B-cell ontogeny [20].

TABLE I. Summary of Laboratory Findings

Markers	1 Patient	2 Patient	3 Patient
CD19	−	+[a]	−
CD20	−	+[b]	+[a]
CD24	+[b]	ND	ND
Kappa/lamda light chain	+/−[b]	+/−[a]	ND
CD57	ND	ND	+[b]
CD10	+[b]	ND	−
t(2;13)(q35;q14)	−	+	+
MyoD	+[b]	+[b]	ND

+ = positive staining; − = negative staining; ND = not determined.
[a]Flow cytometry.
[b]Frozen section immunohistochemistry.

Cytogenetic studies on RMS have indicated that there are distinct karyotypic differences between alveolar and embryonal RMS, so that detection of the t(2;13) (q35;q14) may allow more precise diagnosis of alveolar RMS. Two of our patients (Table I) had the t(2;13) (q35;q14) reported in alveolar RMS [21,22]. Recently, the t(2;13) (q35;q14) was reportedly seen in a single case of B-prolymphocytic leukemia [23]. The t(2;13) (q35;q14) fuses the paired-box homo-domain region of the *PAX3* gene on chromosome 2 with a forehead-domain gene *FKHR* on chromosome 13 [24]. The *PAX3* and the *FKHR* genes belong to different families of developmentally regulated transcription factors [25]. *PAX3-FKHR* fusion protein has been shown to function as a potent aberrant transcriptional activator factor [25] that may activate or inactivate specific targets genes, resulting in deviant antigen expression.

Retrospective study of two of our patients (Patients 1 and 2) revealed positive nuclear staining with MyoD1 antibodies. Immunohistochemistry was also positive for IgM in Patient 1, Kappa light chain was positive by tissue immunostains in Patient 1, and flow cytometry was positive in Patient 2. MyoD1 belongs to a MyoD family of regulatory proteins that is actively expressed during embryogenesis and transactivates genes that encode muscle-specific proteins [26]. The MyoD proteins, characterized by a helix-loop-helix structure with an attached basic regions (bHLH), are part of a large family of transcriptional activators that operates by means of competitive inhibitory and excitatory effects on the promoter regions of the DNA molecules to which they attach [26]. All HLH proteins that have been shown to bind DNA share the ability to bind to a conserve DNA sequence referred to as E-box [26–28]. E-boxes have been identified within the central regions of a wide range of cell type-specific genes, including the immunoglobulins [29]. It is tempting to speculate that oncogenic events in RMS, such as chromosomal translocations, might alter DNA binding of MyoD proteins with transactivation of other type-specific genes that contain E-box, such as the immunoglobulin genes.

The findings in the three patients that we report emphasize the potential expression of CD molecules in primitive undifferentiated rhabdomyosarcomas. The lack of conventional muscle markers and the expression of CD lymphoid markers in a primitive undifferentiated sarcoma should be used for diagnostic exclusion with caution. Additional studies, such as cytogenetics or molecular DNA studies, might be required to arrive at a specific tumor type diagnosis.

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